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Review article on the study of artificially induced ovarian torsion and its effects in rat model

Debopriya Ghosh*, Sandeep Mukharjee,

Department of Physiology University of Calcutta, West Bengal, India.

ABSTRACT

Ovarian torsion is thought to account for one of the major gynaecological surgical emergencies and is the fifth most common surgical emergency after ectopic pregnancy. As the prevalence of ovarian torsion going day by day, it is important to know that what are the reasons behind this disease, and if possible to avoid them. The objective of this experiment is to review pathophysiology of ovarian torsion. In this shows that, there are increase in oxidative stress markers SOD, CAT (catalase) and decrease in MDA level in ovarian tissue of I/R animal model than the control. The histopathological changes such as vascular congestion, edema, haemorrhage, and follicular degeneration were found to be increased in the I/R group compared to the control group.

Keywords: antioxidant enzymes; stress parameters, ovarian torsion.

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Corresponding Author

Name: Debopriya Ghosh
Email: debopriyaghosh29aug1993@gmail.com
Contact: + 91- 84200 62048

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INTRODUCTION

Ovarian torsion is an infrequent gynaecologic emergency with a prevalence of 2.7%, which denotes the bending of the ovary and fallopian tube around the broad ligament. 70% of the cases are of women of reproductive age. Early diagnosis and treatment is necessary for the preservation of the affected ovary, and hence that of fertility, as long-term arterial, venous or lymphatic obstruction of the ovary causes a critical reduction in tissue blood flow and permanent tissue damage [16]. The aim of ischemia treatment is not only to achieve blood flow but also to restore tissue reperfusion. Following ischemia, when the circulation and reperfusion is maintained, a new physio-pathological process called 'reperfusion

injury' is encountered, and this causes several degrees of tissue damage. The total damage that the tissue incurs is the sum of that caused by both ischemia and reperfusion [29], consequently, the prevention of reperfusion injury increases the success of any treatment. Ischemia and reperfusion results in the production of reactive oxygen species (ROS) in tissues such as brain, heart and muscle [35], subsequently, the ROS cause damage to the cell membranes [11]. Along with that ROS causes damage to DNA and mitochondria via lipid peroxidation and cytokine production from activated neutrophils and ultimately lead to tissue damage. Cells have different mechanisms against oxidative damage. These mechanisms involve enzymatic and non enzymatic defence systems. SOD, CAT, and GPx are included in enzymatic defence systems [28].

If the cells do not have enough antioxidant enzymatic activity, ROS levels increase. Therefore, several anti-inflammatory and antioxidant free radical scavenger agents have been used to protect the tissues against I/R injury [6]. Thus, if the impact of these detrimental ROS could be limited, ischemia-reperfusion injury could be avoided or reduced.

Vasoconstriction or microcirculatory disturbance induced by superoxide-mediated endothelial cell dysfunction has been considered one of major causes of organ injury associated with ischemia and subsequent reperfusion. Nitric oxide (NO) has various protective effects on cells during I/R injury. NO has been demonstrated to inhibit oxidative stress and cytokine release [25]. Previous studies have demonstrated that a reduction of NO during hepatic IR injury, generally via a reduction in endothelial nitric oxide synthase activity, leads to liver injury [19].

Previous reports have also suggested that administration of nitric oxide (NO) prior to reperfusion attenuates the consequences of ischemia/reperfusion on cardiac muscle. Various reports indicate that pre-treatment with drugs that enhance NO release protect against ischemia/reperfusion injury.

Collectively, these observations suggest that the development of new therapeutic approaches to control IR injury may be aided by a better understanding of the defence mechanisms that occur organs when it is subjected to ischemic insults. As NO availability has been shown to be beneficial in various experimental models and may be useful in ameliorating ovarian injury by improving the microcirculation. In the present study, we tested the hypothesis that NO enhancement would reduce ischemia and reperfusion injury of the ovary as well.

Ovarian torsion is the twisting of an ovary on its ligamentous supports and can result in a compromised blood supply. Adnexal torsion is a term that is inclusive of either the ovary, fallopian tube, or both. Concomitant ovarian and tubal torsion has been shown to occur in up to 67% of cases of adnexal torsion [3]. Ovarian torsion occurs far more commonly during pregnancy than in the non-pregnant state. Torsion of a 'normal' ovary is a rare event, mostly occurring in childhood [13]. The typical presentation is a unilateral torsion of a pathologically enlarged ovary.

Aetiology of ovarian torsion: Torsion is the total or partial rotation of the ovary around its vascular axis. In the early stages, continued arterial flow with blockade of the venous and lymphatic channels sometimes results in enlargement of the ovary and this can occasionally be massive. If the torsion remains undiagnosed or untreated, arterial stasis can lead to haemorrhagic infarction and necrosis of the ovary. Adnexal torsion almost always involves both the ovary and fallopian tube and isolated ovarian torsion is rare. The mobility of the left ovary tends to be limited by the sigmoid colon, hence about two thirds of adnexal torsions are right sided. Certain anatomical variations and factors have been identified as indicative of risk for ovarian torsion. These include:

Developmental abnormalities: an excessively long fallopian tube or absent mesosalpinx may predispose to torsion.

Ovarian masses: in over half of cases of ovarian torsion an underlying ovarian tumour is present. Malignant tumours are less likely to undergo torsion due to the presence of cancerous adhesions that fix the ovary to the surrounding structures. Thus, tumours that have undergone torsion are most likely to be benign, with dermoid tumours the most

Commonly implicated

Pregnancy: enlarged corpus luteum cysts and the laxity of supporting structures in pregnancy predisposes to torsion. The rate of torsion increases by five times during pregnancy. The corpus luteum regresses in the second trimester, hence the risk of torsion is greatest in the first trimester and decreases thereafter.

Assisted conception: the induction of ovulation during infertility treatment can lead to theca lutein cysts and expansion of the ovarian volume predisposing to torsion.

Previous pelvic surgery: previous pelvic surgery, especially tubal ligation, can have an increased risk of torsion, although the mechanism for this remains unclear.

Incidence: American studies have reported that ovarian torsion is the fifth most common surgical emergency in gynaecology and most reported cases occur in the early reproductive years. The median age reported in a large review was 28 years and about three quarters of patients were aged less than 30 years. An Australian series of 52 cases, published in 2005, reported the median age of patient with confirmed ovarian torsion was 33 years. [34]

Clinical and Epidemiologic Features Ovarian torsion: Clinical and epidemiological features of ovarian torsion can occur in females of all ages; however, women in their reproductive years have the highest prevalence, with 17%–20% of cases occurring in pregnant women[15]. This is probably due to the increased occurrence of physiologic and pathologic ovarian masses, therapy for infertility, and pregnancy compared with that in females at the extremes of age. Symptoms of ovarian torsion are often nonspecific, making it difficult to differentiate from other causes of acute abdominal pain. The classic presentation includes sharp, localized right or left lower abdominal pain and tenderness with a palpable abdominal mass and peritoneal signs [33]. Waves of nausea and vomiting as well as pyrexia have been observed. In some cases, patients experience intermittent pain, making the diagnosis even more challenging [33].

The typical clinical history in ovarian torsion is of the sudden onset of severe, unilateral, lower abdominal pain that worsens intermittently. About a quarter of patients report bilateral lower quadrant pain. Nausea and vomiting are seen in about 70 per cent of cases. Necrosis of the ovary may lead to late findings of pyrexia, tachycardia and hypotension. Two-thirds of patients will have a unilateral adnexal mass on clinical examination with tenderness to palpation in a third of cases. However, the absence of tenderness does not rule out torsion. Peritoneal signs may

indicate advanced disease and are found in under five per cent of cases [13].

Pathophysiologic Features: Initially, the twisted vascular pedicle in the suspensory ligament of the affected ovary compromises venous and lymphatic outflow. However, arterial inflow is sustained because arteries have thick, muscular walls and are less collapsible. This results in diffuse ovarian edema and enlargement, which over time cause the capsule to stretch and increase pressure on the ovary. Arterial thrombosis and ultimately ischemia and infarction ensue. If torsion is left untreated, systemic infection and inflammation may occur [23]. With incomplete torsion, capillary hydrostatic pressure remains increased and obstructs lymphatic drainage, causing massive ovarian edema [33].

Predisposing Conditions: Large, heavy cysts and cystic neoplasms, such as benign mature cystic teratomas, hemorrhagic cysts, and cystadenomas, commonly predispose the ovary to swing on its vascular pedicle [20]. The large cystic ovaries seen in ovarian hyperstimulation syndrome are another predisposing factor for torsion [20]. Conversely, it is rare to see ovarian torsion from cysts smaller than 5 cm [32]. Torsion of a normal ovary is unusual but is more common in adolescents. Postulated causes of normal adnexal torsion include markedly mobile fallopian tubes or mesosalpinx, elongated pelvic ligaments, fallopian tube spasm, strenuous exercise, or abrupt changes in intrabdominal pressure. Ovarian torsion is uncommon after pelvic inflammatory disease, endometriosis, or malignant neoplasms; this may be due to the presence of adhesions, rendering the ovaries relatively immobile [33,23]. Some studies have shown that the right ovary is more likely to twist because the space occupied by the sigmoid colon on the left side protects the left ovary [3,33].

Investigations: Ovarian torsion is primarily a clinical diagnosis based on a thorough history and meticulous examination. Maintaining a high index of suspicion is important. Laboratory tests are often unhelpful in trying to verify a diagnosis of ovarian torsion. However, they can assist in ruling out alternative or co-existing diagnosis of lower abdominal or pelvic pain. Therefore, the following tests should be performed:

- Beta HCG should be performed to diagnose a concomitant pregnancy.
- Full blood count: an elevated white cell count is a nonspecific finding and is rarely of value. Haemorrhage may result in anaemia.
- Electrolyte imbalances may be seen in severe cases where there has been persistent vomiting.

Some authors have also associated ovarian torsion with increased serum levels of interleukin-6, but

further research is needed to establish this serum marker as a diagnostic tool.

Ultrasound is the most important investigation for patients with suspected ovarian torsion. However, in the second and third trimesters of pregnancy, it is sometimes difficult to visualise the ovaries as they are displaced from the pelvis by the enlarging uterus. Some of the pelvic sonographic features found in cases of ovarian torsion are:

- i. Heterogeneously enlarged ovary.
- ii. Presence of peripheral follicles.
- iii. Midline ovary.
- iv. Free fluid in pouch of Douglas.
- v. Twisted pedicle leading to 'whirlpool sign' (uncommon).
- vi. Asymmetric thickening of ovarian wall cysts.

Colour Doppler in ultrasound is commonly used, but may not be diagnostic of torsion, depending on the degree of torsion and the sometimes subacute nature of the pathology. The presence of blood flow shows that the ovary may be still viable, but does not rule out ovarian torsion. Greyscale assessment of the ovary combined with Doppler imaging may enhance the diagnostic accuracy. Colour Doppler imaging often shows an enlarged ovary with absent parenchymal perfusion. CT and MRI modalities have limited use in the diagnosis of torsion. They may demonstrate an adnexal mass or enlarged ovary, but provide no information on the blood flow to the involved ovary. However, they may be more helpful in ruling out other differentials of pelvic pain in case of diagnostic uncertainty and in the presence of pelvic mass.

Management Of Ovarian Torsion: The main principle of treatment is timely surgical intervention to preserve ovarian function. Initial management involves stabilising the patient and ruling out other causes of acute abdominopelvic pain (such as ectopic pregnancy, pelvic inflammatory disease, pyelonephritis, appendicitis, endometriosis and, rarely, degenerating leiomyomata). A swift surgical evaluation is required in most of the cases presenting as acute abdomen. Salpingo-oophorectomy may be performed if tissue necrosis, severe vascular compromise or peritonitis is obvious. Oophorectomy is not required in all cases, particularly when laparoscopic assessment is performed early. This approach has the potential to uncoil the torqued ovary and anchor it with a possible oophoropexy. Some authors have suggested oophoropexy on the contralateral side might be considered in young patients needing oophorectomy for an ovarian torsion. Intra-operative assessment of the ovary in question is crucial in determining its potential viability. The determination of whether an acutely torqued ovary is viable can be difficult. With the appropriate equipment, it is possible to use

intravenous fluoresceine and to view the ovary with an ultraviolet light source. However, this is not possible with laparoscopy and remains a technique applicable only to open surgery. Early conservative management is preferred with the 'success' rate of ovarian preservation of 88 per cent reported by multiple studies. Management of torsion in pregnancy is similar to non-pregnant women, but often technically more difficult due to the mass effect of the gravid uterus.

Cellular microenvironment and changes in amount of reactive oxygen species in ovarian torsion: Reactive oxygen species (ROS), derived from molecular oxygen, may present as free radicals or other forms; they have electronically unstable and ionized atomic structure, which interacts with biological macromolecules by capturing electrons and interfering with their biological functionality [30]. There are several enzymatic activities involved in the generation of ROS: the reduction of dioxygen (O₂) in the mitochondria can lead to various intermediate forms of ROS; within peroxisomes, the reduction of amino acid oxidase triggers the oxidative deamination in the α -keto acids and copper and iron ions generate ROS that may be released in to the blood flow by transferrin, albumin, and ceruloplasmin.

Finally, as well as intracellular nicotinamide adenine dinucleotide phosphate [NAD(P)H]-oxidase, cyclooxygenase, nitric oxide synthase (NOS) and xanthine oxidase produce ROS [24]. Reactive nitrogen species (RNS) include nitric oxide (NO), nitrogen dioxide (NO₂) and non-reactive species such as peroxynitrite (ONOO⁻), and nitrosamines [27]. In mammals, RNS are mainly derived from NO, which is formed by NOS from O₂ and L-arginine using NADPH as an electron donor [17], and from its reaction with the superoxide anion, which forms peroxynitrite [5]. Peroxynitrite could trigger lipid peroxidation and nitrosation of several tyrosine molecules, which play a key role in enzyme function and signal transduction pathways. Moreover, accumulating evidence suggests that the activity of endothelial NOS (eNOS) is increased in response to the LH surge and hCG [12]. Moreover, animal models showed that eNOS expression is enhanced during chronic hypoxic conditions [8].

Conversely, it has been demonstrated that hypertension in humans may occur due to suboptimal vascular endothelial production of NO [31]. The physiological function of ROS has been widely studied in monocyte-macrophages, granulocytes, natural killer (NK) cells and neutrophils. Within these cells, there is a clear evidence that the NAD(P)H oxidase, the myeloperoxidase and the NOS produce anion superoxide, hypochlorous acid and nitrogen monoxide, triggering the cytotoxic responses of the immune system [24]. Furthermore, oxidizing agents

play a key role in the phosphorylation and activation of protein kinases, and in the phospholipase signalling pathways mediated by Ca⁺⁺. In this regard, oxidative stress activates phosphorylated proteins. Considering the point that the activity of these protein kinases are strictly involved in the cell cycle regulation, it has been showed that the inhibition of these transduction cascades by ROS scavenger and antioxidant agents, such as superoxide dismutase (SOD), can inhibit the cell cycle progression [26].

Markers Of Oxidative Stress: Causes And Effects: Since ROS are potentially harmful, the human body has evolved highly complex antioxidant defense systems, both enzymatic and non-enzymatic, which synergistically function in combination. Some antioxidants are produced directly in the intracellular microenvironment (enzymatic antioxidants), such as glutathione oxidase and peroxidase, ubiquinone (CoQ10), α -lipoic acid (ALA), SOD and superoxide catalase [2], while the non-enzymatic antioxidants consist of dietary supplements and synthetic antioxidants such as vitamin C, glutathione, taurine, hypotaurine, vitamin E, Zn, selenium (Se), betacarotene, and carotene [22]

Mechanism of antioxidants action depends on their concentration, which is variably presented in fluids and tissues. Multiple redox reactions occur within cell metabolism which can lead to oxidative stress if unbalanced in homeostatic mechanisms. Although cells have several intrinsic antioxidant mechanisms, when ROS are present in large amount, the ability to rebalance homeostasis is exceeded and as a result cellular damage may occur [22]. An excess of ROS may cause a cascade of events such as the release of Ca⁺⁺ which results in mitochondrial permeability and provokes mitochondrial membrane instability and consequent cessation of adenosine triphosphate (ATP) production; the lipid peroxidation, which increase the peroxy radicals, damage of amino acids which leads to the formation of carbonyl groups. Oxidation of the mitochondrial DNA (mtDNA) without protection of histones, does not own any repair mechanisms [5]. As result of this complex mechanisms, oxidative stress finally causes DNA damage and/ or apoptosis of the cell (Lee JY et al.,2010). The total antioxidant status (TAS) was employed to assess the general ant oxidative status [9].

Accordingly, total oxidant status (TOS) is obtained to ascertain the overall oxidation status. Represented as the ratio of TOS to TAS, the oxidative stress index (OSI) considered a precise index of oxidative stress [10]. Oxidative stress seems to play a key role in the physiologic processes of menstrual cycle and ovulation, embryo implant, placental framework development, menopause [2]. Conversely, accumulating evidence suggest that a breakdown in red-ox homeostasis occurs during several

reproductive diseases such as endometriosis, polycystic ovary syndrome, unexplained infertility, spontaneous abortion, recurrent pregnancy loss, preeclampsia, intrauterine growth restriction and preterm labor. In particular, this review will try to shed light on the redo-ox process during the adnexal torsion in paediatric and adolescent patients.

DISCUSSION

Ovarian torsion is a serious gynaecologic emergency condition. Although it might affect all women, it is usually seen in women of reproductive age [4]. Ovarian ischemia is the result of torsion and leads to cell death because of insufficient perfusion of the tissue [14]. A reduction in ovarian blood flow may lead to ovarian necrosis, ischemia, and infarction. Early diagnosis and treatment of ovarian torsion are crucial for the protection of ovarian function. Moreover, a delay in the diagnosis and treatment may cause ovarian loss and infertility. Ischemic tissues need to recover blood supply for regeneration of cells and disposal of toxic metabolites. The primary pathophysiologic mechanism in ovarian torsion is ischemia followed by reperfusion that causes ischemia-reperfusion (I/R) injury in the ovaries. However, reperfusion of the ischemic tissue paradoxically leads to much more serious damage to the tissue than the damage caused by ischemia [1]. The ischemia and reperfusion injuries are related to the production of reactive oxygen species (ROS) [7]. It was previously shown that Reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radicals are formed after reperfusion. Reactive oxygen species cause DNA damage, cell membrane, and mitochondrial damage via lipid peroxidation and cytokine production from activated neutrophils and ultimately lead to tissue damage.

Ischemia leads cells to rapidly manifest distinct biochemical, structural, and functional alterations causing ovarian dysfunction. Oxidative damage is thought to play an important role in I/R injury. It has been suggested that I/R triggers a series of reactions mainly in the organ that is clamped and reperfused, and these reactions elicit a systemic inflammatory response by the release of cytokines and inflammatory mediators (tumour necrosis factor, interleukin-6, platelet-activating factor, leukotrienes, and NO) that cause the formation of oxygen ROS with consequent oxidative stress [6]. Toxic metabolites such as MDA and ROS are increased due to ovarian torsion. This ischemic process is dangerous for cells because of increasing toxic molecules. Lipid peroxidation, as a free radical-generating system, has been suggested to be closely related to I/R-induced tissue damage, and MDA levels may represent a good indicator of the rate of lipoperoxidation. MDA, which is a metabolite of the ROS-mediated lipid peroxidation cascade, is a highly toxic molecule and shows oxidative stress. MDA is a marker of tissue injury and also induces destruction of

polyunsaturated fatty acid in the cell membrane. MDA level significantly increases in I/R injury, suggesting wall fluidity and permeability of the damaged cell. Consistent with this previous observations, results obtained in our present study confirm that tissue MDA levels significantly increase in postischemic reperfusion. Our results also demonstrated that administration of L-arginine has caused a significant decrease in MDA levels, suggesting the protective effect of the drug may be due, in part, to its scavenger capacity. Cells protect themselves from ROS injury via intracellular enzymes such as SOD, catalase and GSH. There have been several reports which proved the fact that ischemic tissue responds by raising the activities of SOD, CAT, and GSH to avoid the harmful effects of toxic substances. Catalase is an antioxidant enzyme that scavenges the cell from pro-radical hydrogen peroxide (H_2O_2). Glutathione provides major protection in oxidative injury by participating in a cellular system of defence against oxidative damage. Several reports have indicated that tissue injury induced by various stimuli are coupled with glutathione depletion [18], and the maintenance of high levels of glutathione is essential to reduce oxidative stress during organ re oxygenation, therefore, the decrease in glutathione levels during I/R was probably due to its consumption during oxidative stress. Study shows that oxidative stress involvement was confirmed by a decrease in GSH level in I/R groups with respect to the control group. Similarly, catalase was used for the determination of antioxidant level. The results revealed that catalase significantly increased in the group III (I/R+ arginine) compared with Group II (I/R injury). Studies also shows that a decreased SOD level in the I/R group compared to the sham operated group. MDA level are significantly higher in I/R group than the control group. SOD, CAT, and GSH activities were significantly higher in I/R group than Control Group. Studies shows that the histopathological changes such as vascular congestion, edema, haemorrhage, and follicular degeneration were found to be increased in the I/R group compared to the control group.

CONCLUSION

Ovarian torsion a common gynaecological emergency which need proper early diagnosis and treatment. The main objective of this study is to evaluate and monitor the protective effect of arginine in ischemic /reperfusion tissue injury of ovary. This study shows that the aetiology of Ovarian torsion and the pathophysiology of Ovarian torsion for the future studies to develop the counter measures. My study shows that, there are increase in oxidative stress markers SOD, CAT (catalase) and decrease in MDA level in ovarian tissue of I/R animal model than the control. The histopathological changes such as vascular congestion, edema, haemorrhage, and follicular degeneration were found to be increased in the I/R group compared to the control group.

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